EPA
www.epa.gov/research
science in ACTION
INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

Effect of Changing Treatment Disinfectants on the Microbiology of Distributed Water and Pipe Biofilm Communities using Conventional and Metagenomic Approaches

Risk Management Research Project - Addressing Drinking Water Challenges through Science and Innovation

Background & Problem

Drinking water distribution systems are a potential source of waterborne illnesses from the growth of specific pathogens associated with larger microbial communities, known as biofilms, which occur on drinking water pipe surfaces. *Mycobacterium avium*, *Legionella pneumophila*, and *Naegleria fowleri* are examples of these pathogens that are currently listed as priority contaminants on the EPA's Contaminant Candidate List 3. Previous studies have demonstrated that the viability and virulence of these pathogens is influenced by other microbes in the biofilm communities; therefore, a better understanding of the entire range of microbes in the biofilms is needed to develop pathogen control strategies. Current treatment includes the use of halogen disinfectants, such as chlorine and monochloramine. Chlorine is the most commonly used drinking water disinfectant in the U.S., but the sustainability of this treatment has been questioned due to the potential for carcinogenic chlorinated byproducts. Because of this, many utilities are now using monochloramine as both the primary and residual disinfectant. Previous studies have documented the differences between the effectiveness of chlorine and monochloramine at killing specific types of pathogens and the ability to penetrate into biofilms; however, there is limited data on their effectiveness at controlling the occurrence and growth of pathogens in distribution systems. In addition, the impact to the overall microbial community composition by these disinfectants is also unknown.

Purpose of the Studies

The purpose of this research was to add to our knowledge of chlorine and monochloramine disinfectants, with regards to effects on the microbial communities in drinking water distribution systems. A whole metagenome-based approach using sophisticated molecular tools (e.g., next generation sequencing) was used to assess the microbial composition and the metabolic potential in these communities. In addition, this research produced insights on the importance of certain operational parameters in biofilm-related research.

Results and Conclusions to Date

Study 1 The implementation of metagenomic tools has provided a robust dataset on the diverse and highly complex nature of drinking water pipe biofilm communities. Metabolic pathways[1] and sequences assigned to a variety of pathogen-related functions (e.g., virulence factors and antibiotic resistance) were identified in both chlorine and monochloramine treated drinking water samples. The results showed differences in community structure and function among communities exposed to the disinfectants:

- Genes indicative of *Legionella* were more abundant in the free chlorine treated samples.
- Genes indicative of *Mycobacterium* were more abundant in monochloramine treated samples.
- Although genes that code for proteins needed to survive/avoid/repair oxidative damage were identified in both the chlorine and monochloramine treated communities, exopolysaccharide (EPS)[2] genes of *Mycobacterium* were more abundant in communities from monochloramine treated water, which likely translates to more biofilm material.
- Occurrence of antibiotic resistance was not preferential to either treatment; however, protein groups associated with specific oxidant defense mechanisms were more abundant in one or the other treated systems[3].

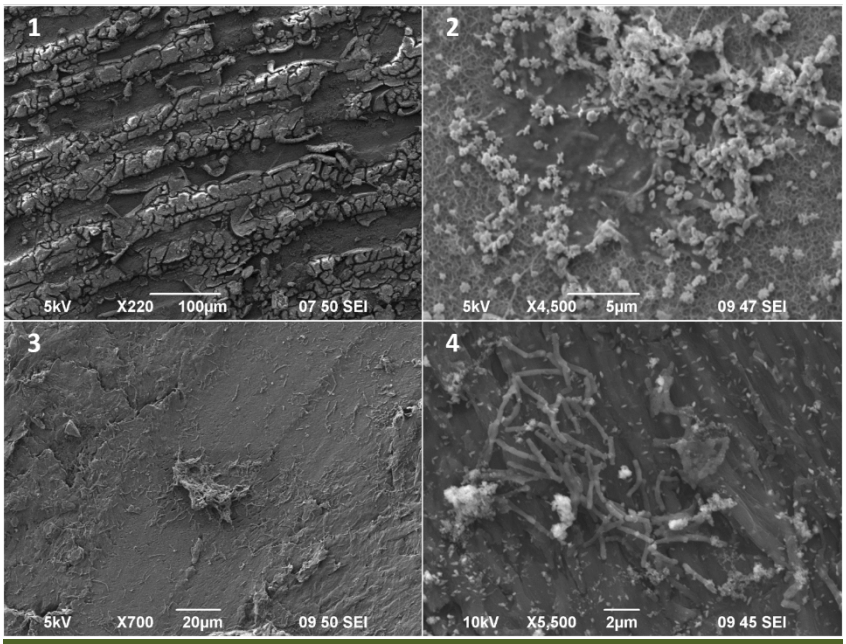
[1] As evidenced by specific gene sequences that potentially code for the enzymes needed to complete complex biological transformations.

[2] *Mycobacterium* is one group of bacteria that produce a significant proportion of the EPS in biofilms. EPS serves to protect biofilm organisms from direct exposure to disinfectants and to promote microbial adhesion to surfaces and overall biofilm formation.

[3] Example: glutathione reductase and thioredoxin reductase, which collectively code for proteins needed to protect other proteins from oxygen radicals, were more abundant in the chlorine samples. In contrast, the dps gene, needed to protect DNA from oxidation damage, was more abundant in monochloramine samples.

The data from Study 1 suggest that disinfection treatments can influence the occurrence of specific types of gene systems in pipe biofilm communities. The impact of these differences on the occurrence and virulence of waterborne pathogens needs to be the subject of continuing future research.

Publication Available:
Gomez-Alvarez V., Revetta R.P., Santo Domingo J.W. (2012). Metagenomic analyses of drinking water receiving different disinfection treatments. *Applied Environmental Microbiology*, *78*(17):6095-6102. (DOI:10.1128/AEM.01018-12)



Electron microscope images of biofilm growth on copper (images 1 & 2) and PVC (images 3 & 4) drinking water pipe material from the studies.

Study 2 A molecular approach was used to characterize biofilm communities formed in monochloramine treated drinking water. Samples of communities that formed on various types of biofilm capture devices were evaluated to determine if they differ in their particular community structure and if the compositions vary over time as the biofilms form. Overall the results supported one of the observations of Study 1: *Mycobacterium* was a dominant bacterial group in biofilms from the monochloramine treated waters. However, this study expanded on the observation by showing that these slow-growing *Mycobacterium* are not the main initial biofilm colonizers. Their predominance after two months suggests that they become integrated into the communities as persistent secondary colonizers of biofilms. In general, the most significant variable that influenced the composition of biofilm communities was the age of the biofilm and not the type of surface material. This observation has important practical implications for future biofilm sampling schemes and for identifying potential management strategies.

Publication Available:
Revetta R.P., Gomez-Alvarez V., et al. (2013). Establishment and early succession of bacterial communities in monochloramine-treated drinking water biofilms. *FEMS Microbiology Ecology, 86*(3):404-414. (DOI: 10.1111/1574-6941.12170)

Study 3 A current study of the operational parameters of a simulated chloraminated drinking water distribution system was designed to reflect three distinct operational conditions of a typical distribution system: (1) normal, (2) failure leading to nitrification, and (3) restoration via a chlorine burn. Preliminary results show little variation in biomass in bulk water and biofilm samples during the normal operation. However, an increase in biofilms was detected when the concentration of chlorine residual was decreased in the system, which lead to nitrification conditions and failure of the system. Biomass levels decreased after a chlorine burn. Analysis of the biofilms showed clustering of samples and significant differences in functional and community structures during these different operational conditions. For example, environmental *Mycobacterium*-like sequences peaked during normal operations but decreased after failure of the system, while the abundance of nitrifying bacteria increased as nitrification increased. Data on the impact of the chlorine burn has yet to be analyzed. The results of this study will be submitted for publication in 2015.

Expected Outcome

The metagenomic approaches used in these studies will help to develop a more comprehensive understanding of the microbial ecology of drinking water distribution systems relative to what disinfectants are used. Such information is critical to the design of effective management practices and ultimately helps to prevent waterborne disease and safeguard human health.

Principal Investigators

Randy Revetta
(513) 569-7129
revetta.randy@epa.gov

Vicente Gomez-Alvarez
(513) 569-7362
gomez-alvarez.vicente@epa.gov